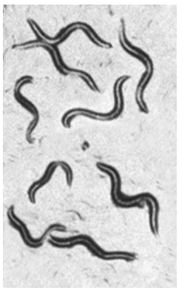

The genome sequence of *Caenorhabditis elegans* is due to be completed around the end of this year. *C. elegans* will therefore be the first animal to have its genome completely sequenced. To mark this outstanding achievement, we are publishing a series of articles celebrating worm genetics. Articles will review the contributions that worm genetics has made to fundamental aspects of biology such as cell death, signal transduction, sex determination and neurobiology.

Genetics of RAS signaling in *C. elegans*

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Genetic analysis of the RAS function in Caenorhabditis elegans has not only clarified the functional relationship of signal transduction proteins, but also led to the discovery of new proteins involved positively or negatively in RAS signaling. The stereotyped development of C. elegans has allowed many of the functions of RAS to be elucidated at the level of fates of individual cells.

Analysis of RAS function in *C. elegans* has revealed many aspects of signal transduction and development and contributed greatly to our understanding of a universal RAS signaling pathway and some of its variations. RAS was discovered for its role in many human cancers; elucidation of the mechanisms by which RAS exerts its effects and is regulated has continued to be a major goal of basic cancer research[1]. Here, we review the genetics of *C. elegans* RAS and the implications of these studies. The analysis of RAS also illustrates the way in which *C. elegans* genetics is done. With the impending completion of the genomic sequence and the increasing number of tools for the analysis of gene function, many of the complex regulatory circuits and networks will be worked out in *C. elegans*. The success with elucidating RAS function inspires the future of this effort.

Discovery of *let-60* by genetic screens

The developmental role of RAS in *C. elegans* was discovered by analyzing one aspect of development, the formation of the vulva during larval development. Intensive genetic and developmental characterization of vulval development set the stage for the studies of RAS discussed here[2]. The *C. elegans* vulva forms from the descendants of three precursor cells induced from a field of six potential precursors (vulval precursor cells or VPCs; Fig. 1). In the absence of an inductive signal, all six VPCs have the 3° VPC fate: they undergo a single round of mitosis and their progeny fuse with the epidermal syncytium that envelops most of the worm. In the presence of the inductive signal, three central VPCs undergo a total of three rounds of mitosis and their progeny differentiate to form the mature vulva. The induced VPCs are of two types, 1° and 2°, which are distinguished by the types of progeny cells they produce; molecular markers for these cell fates are just now becoming available (e.g. Ref. 3). Mutants that lack induction in which all six VPCs have the 3° fate exhibit a vulvaless (Vul) phenotype (Fig. 2). Mutants that have excessive vulval induction – either because there is excessive signaling or the VPCs do not require an inductive signal for vulval development – exhibit a multivulva phenotype (Muv). 1° and 2° fates indicate RAS activity while 3° fates indicate lack of RAS activity, as we shall see. Thus the extent of vulval differentiation allowed a fairly direct readout of RAS activity (Fig. 1).

RAS is encoded by the *let-60* gene. Baillie and his colleagues[4,5] found the first *let-60* mutations as recessive lethal mutations on chromosome IV in an effort to saturate the genetic map of essential genes – hence the name *let*, for lethal. Subsequently, most of the other alleles were obtained and analyzed based on their effects on vulval development. More than 30 *let-60 ras* mutations were isolated in several genetic screens. The mutant alleles of *let-60 ras* can be divided in three general classes: loss-of-function (lf), gain-of-function (gf), and dominant negative (dn) mutations. Table 1 summarizes the phenotypes of some of the properties of *let-60 ras* mutations. Nine dn mutations were isolated as suppressors of the multivulva phenotype of *lin-15(lf)* mutations[6–8]; *lin-15* is a negative regulator of vulval induction (see below). Additional recessive alleles of *let-60 ras* were also obtained as suppressors of the multivulva phenotype of *lin-15* or as intragenic revertants of *let-60(dn)* (Refs 6, 7). Five gf alleles with the identical G13E change were found as multivulva mutants from a wild-type strain[9] (previously called *lin-34*), suppressors of the vulvaless phenotypes of *let-23(lf)* (Ref. 10), of *lin-10(lf)* (D. Parry, S. Kim and R. Horvitz, cited in Ref. 6), of *let-60(dn)* alleles[7] and of *let-341* (S. Clark and R. Horvitz, cited in Ref. 6). A temperature-sensitive *let-60(gf)* allele (L19F) was isolated more recently in a screen for mutants with abnormal vulvae[11].

Because a null and several of the lf mutations in *let-60 ras* cause an early larval lethality, the functions of

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let-60 ras in vulval induction as well as in some other cell differentiation and migration events were revealed by using dominant mutations or partial lf mutations. The biology of a given gene is often revealed using non-null mutations, a valuable point to stress as we approach a post-genomic sequencing project era. Classical forward genetics will be as useful as ever for isolating such special alleles because genetic screens can select out relatively rare, but informative, mutations. Of course, rare alleles can also be misleading and need to be interpreted in the context of a complete genetic analysis, including knowledge of null phenotype.

let-60 ras was cloned based on its genetic map position and found to encode the *C. elegans* homolog of *bona fide* RAS proteins[12]. The 184-amino acid LET-60 protein is extremely similar in overall sequence to all three mammalian RAS proteins. However, LET-60 RAS from *C. elegans* as well as from another nematode, *Pristionchus pacificus* (Ref. 13), is more similar to K-ras and N-ras than to H-ras. Such a distinction is important to keep in mind: study of the LET-60 RAS pathway might reveal some functions and features that are specifically related to a subset of the mammalian Ras proteins. The three *ras* loci in mammals probably have overlapping and distinct functions, including the ability to bind effector proteins. For example, mice deficient in N-*ras* or H-*ras* have no apparent abnormalities, but those deficient in K-*ras* exhibit embryonic lethality and early hematopoietic defects, phenotypes that are exacerbated by a reduction of N-*ras* dosage[14].

At about the same time that *let-60 ras* was cloned, *let-23* was found to encode a *C. elegans* homolog of the epidermal growth factor (EGF) receptor[15]. Construction of doubly mutant strains defective in both *let-23* and *let-60* revealed that LET-23 required LET-60 for its action. *let-60(gf)* mutations bypass the requirement for *let-23* for vulval formation and viability[7,16], and we now know that activated LET-23 is suppressed by *let-60(dn)* mutations; Ref. 17). It was then clear that genes that mutated to produce vulvaless and lethal phenotypes were candidates for encoding other components of the pathway defined by LET-23 and LET-60. Additional loci that produced these phenotypes could be divided into two classes, based on whether or not they are suppressed by *let-60(gf)*. Mutations in *lin-3*, *sem-5* and *let-341* were suppressed by activated RAS and thus, in the simplest view, block upstream of RAS, while *lin-45* was not suppressed by activated RAS, thus blocking downstream of RAS (Fig. 3).

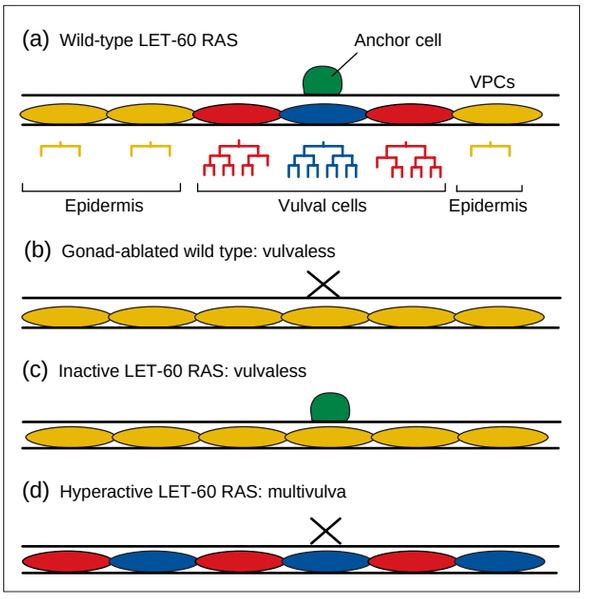

FIGURE 1. Vulval development and effects of RAS mutants. (a) In a wild-type animal, the anchor cell induces the three closest vulval precursor cells (VPCs) to generate the 1° (blue) and 2° (red) vulval lineages rather than the 3° non-vulval lineage (yellow). The 1° and 2° lineages generates subsets of vulval cells while the 3° lineages generate cells that become hyp7 epidermis. (b) In the absence of an anchor cell the animals are vulvaless because all six VPCs generate 3° lineages, thus producing additional hyp7 epidermis at the expense of vulval tissue. (c) Mutants with decreased RAS activity are also vulvaless. (d) Animals with hyperactive RAS are multivulva because up to three additional VPCs generate vulval cells. The difference between 1° and 2° VPCs is fascinating and involves the extent of RAS pathway activity as well as signaling among VPCs (see Ref. 2).

TABLE 1. Properties of *let-60 ras* mutations in *C. elegans*

Alleles	Genotype	Phenotype[a]	Biochemical/genetic properties
Loss-of-function (lf)	*lf*/+	Wild type	Haploid sufficient
	lf/*lf*	L1 lethal	Non-functional protein
	lf/*lf* (partial)	Vulvaless/lethal	Retains some activity, various defects
Dominant negative (dn)	*dn*/+	Vulvaless	dn Proteins might compete with wt protein for an activator. G10A, G15S, G15D, K16N are suppressible by *sur-5* mutations. S89F and D119N are not suppressed by *sur-5* alleles
	dn/*dn*	Larval lethal	
	dn/*dn*(D119N)	Vulvaless	
Gain-of-function (gf)	*gf*/+	Weak Muv	G13E has been isolated multiple times.
	gf/*gf*	Muv	L19F is a Ts gf. L19F/+ is essentially wild type No G12V alleles have been isolated[b]
Multicopies of *let-60* (+)	+/+; +++	Muv	As in mammalian cells, high transgene dosage of wild-type *ras* also causes constitutive activity

[a]Only vulval induction and viability phenotype are considered in this table (Refs 4–9, 11, 12).
[b]Because overexpression of *let-60* is lethal, a strongly activating mutation is presumably also lethal.

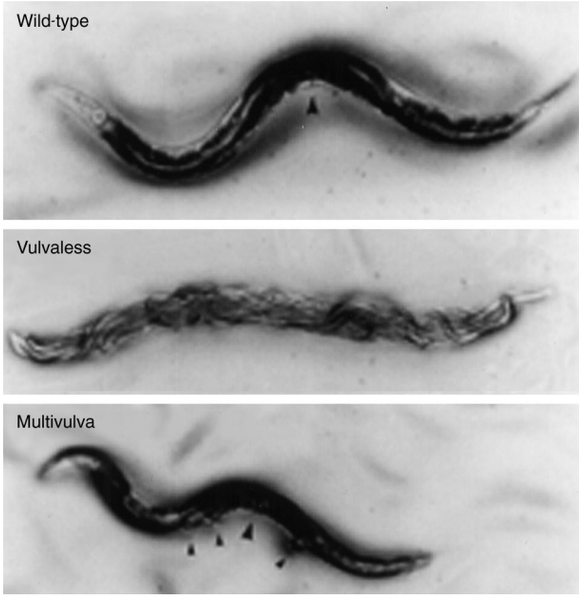

FIGURE 2. Vulval phenotypes of RAS pathway mutants. Photomicrographs of wild-type, vulvaless and multivulva animals, viewed under a dissecting microscope on a lawn of *Escherichia coli* on a Petri plate. These photographs illustrate what the worms look like to the *Caenorhabditis elegans* geneticist.

Factors downstream of RAS

In 1990, when the molecular analysis of the *C. elegans let-60* locus was reported[6,7,12], the targets of RAS in mammalian cell signaling were not known, although RAS was known to have profound affects on cell proliferation. Genetic screens for mutations that suppress the Muv phenotype caused by *lin-15(lf)* mutations or *let-60 ras(gf)* mutations have been extremely effective in identifying downstream genes (reviewed in Refs 18–20). These screens not only identified essential factors acting after *ras*, such as *lin-45* (RAF), *mek*-2 (MAP kinase kinase), *mpk-1* (MAP kinase) and *sur-2* (novel; Refs 21–27), but also identified many other modifiers and regulators such as *ksr-1* and *sur-8/soc-2* (Refs 28–30). The genes *lin-1*, *lin-31* and *lin-25*, previously identified by direct screening for vulval developmental defects[9,16], have also been shown to act downstream of *let-60 ras* in the signaling process[7,31–33].

LIN-1 and LIN-31 are two downstream factors that are probably regulated by MAP kinase phosphorylation. The genes *lin-1* and *lin-31* encode members of the ETS-domain and winged helix transcription factor families, respectively[31,32]. Both gene products act downstream of MPK-1 and are apparently regulated through phosphorylation by the *mpk-1* MAP kinase, although LIN-31 appears to be a vulval-specific factor[34,35]. Phosphorylation of LIN-1 in its C-terminal domain will probably repress its function as an inhibitor of vulval cell fate, because gf mutations that disrupt this region cause LIN-1 to inhibit downstream signaling, thereby interfering with vulval development[35]. LIN-1 and LIN-31 interact directly to form a LIN-1–LIN-31 complex that inhibits vulval formation[34]. LIN-31 also has a positive role because it is necessary for some cells to be induced. Phosphorylation of LIN-31 by MPK-1 disrupts the complex, thus disrupting the inhibitory function of LIN-1. LIN-25 and SUR-2 probably act as positive factors downstream of MPK-1 in vulval differentiation[27,33]. Both are novel proteins of unknown biochemical function. They could act in a branch downstream of *mpk-1*, and have been proposed to act as transcription cofactors, based on their position in the genetic pathway.

Genetic suppressor analyses have also identified several genes that appear to act as regulators of the signaling pathway (Fig. 3). Mutations in many of these genes do not cause an obvious mutant phenotype in vulval development, suggesting that their individual roles in vulval induction are not be essential. The function of these genes in the RAS pathway is revealed by one or more of three genetic characteristics. First, mutations in these genes suppress the phenotype of either activated or dn *let-60* mutations. For example, *ksr-1* and *sur-8/soc-2* lf mutations suppress the Muv phenotype of *let-60(G13E gf)* (Refs 28–30), suggesting a positive role of *ksr-1* and *sur-8/soc-2*. Secondly, overexpression of some genes might enhance or suppress the phenotype of *ras* mutations. For example, overexpression of *sur-8/soc-2* enhances the Muv phenotype of *let-60(gf)*. Finally, mutations of *ksr-1* or *sur-8/soc-2* dramatically enhance the Vul phenotype of partial lf mutations in main linear pathway genes, such as *lin-45 raf* or *mpk-1*, suggesting that these genes play an important positive regulatory role in animals with wild-type LET-60 (Refs 28–30). Furthermore, in a *sur-8/soc-2; ksr-1* double mutant, vulval induction is essentially eliminated, suggesting that the main RAS signaling pathway depends on the activity of these two modifier genes[30]. It is also possible that SUR-8/SOC-2 and KSR-1 act in two parallel sub-pathways. The gene *ksr-1* encodes a novel protein kinase[28,29] with homologs in *Drosophila* and mammals; *ksr-1* probably acts universally in RAS signaling[36]. The *sur-8/soc-2* gene encodes a novel leucine-rich repeat containing protein[30,37] that binds directly to RAS but not to a RAS effector domain mutant[30], suggesting that it could act as an intermediary between RAS and another target, or function to facilitate the interaction of RAS with a target. Human and mouse SUR-8/SOC-2 are able to bind K-RAS and N-RAS but not H-RAS. *sur-8/soc-2* was also identified in genetic screens for mutants that block the effect of FGF-receptor that has been hyperactivated by mutation of the tyrosine phosphatase CLR-1 (Refs 37, 38).

Factors upstream of RAS

Genetic screens have found a number of factors that regulate directly or indirectly RAS activation in *C. elegans* (Fig. 3). The EGF-like growth factor LIN-3 and its putative receptor LET-23 act upstream of LET-60. The discovery that *sem-5* encodes an adaptor protein with SH3 and SH2 domains[39], and the discovery of SOS in *Drosophila*[40,41], led to the biochemical linking of receptor tyrosine kinases to RAS activation. One component missing from *C. elegans* is an exchange factor for RAS. We provisionally assume that there will be such an activator, based on the behavior of dn mutations: dominant interfering mutations of LET-60 RAS interfere with wild-type but not activated RAS, indicating that they compete for an activator rather than an effector of RAS (Ref. 8). One gene, *let-341*, has all the right genetic properties to encode a RAS exchange factor[42]; however, it has not been cloned.

Three genes, *lin-2*, *lin-7*, and *lin-10* positively regulate LET-23 activation of RAS (Refs 43–46). All three genes encode PDZ domain-containing proteins. LIN-2, LIN-7 and LIN-10 form a complex that localizes LET-23 to the correct basolateral membrane, thereby allowing full signaling. An emerging theme in signal transduction is that proteins containing multiple PDZ domains organize signaling complexes; LIN-2, LIN-7 and LIN-10 might represent subunits of such a complex.

The involvement of *ptp-2*, which encodes a homolog of the tyrosine phosphatase SHP2 (*csw* in *Drosophila*), in RAS activation was revealed by a targeted gene deletion[47]. Lack of PTP-2 diminishes the Muv phenotype of activated LET-23 or LET-60, indicating a positive role for this tyrosine phosphatase in the RAS pathway. Whether PTP-2 is a co-factor of LET-23-mediated RAS activation or acts in a parallel pathway is not known.

Genetic screens have also identified a number of negative regulators of RAS signaling[48,49] (Fig. 3). The loci described by Ferguson and Horvitz[48] fell into two classes, A and B, such that mutation both of an A and of a B class locus is necessary to result in RAS pathway activation. Mosaic analysis of two loci, *lin-15* and *lin-37* (Refs 50, 51), suggested that they function in cells other than the VPCs. The gene *lin-15* encodes two nuclear proteins, suggesting that this locus might regulate an intercellular signaling pathway that, ultimately, affects RAS signaling negatively[52,53] (L. Huang, J. DeModena and P. Sternberg, unpublished).

Screens for extragenic suppressors of the *let-23*, *lin-2* or *lin-10* vulvaless phenotypes[10,54,55] identified the previously known *unc-101* locus as well as new loci, *gap-1* and *sli-1* (Refs 8, 49, 50). UNC-101 is a homolog of a *trans*-Golgi clathrin-associated adaptin complex medium chain[54]. It might regulate the subcellular localization of LET-23 or other components of the RAS pathway. GAP-1 is a putative GTPase activating protein for LET-60 (Ref. 55) and its identification as a negative regulator is, thus, especially pleasing because GAPs are biochemically negative regulators of RAS activity. SLI-1 is a homolog of the oncoprotein c-CBL (Ref. 49), which associates with many transmembrane receptors. Studies in *C. elegans* were the first to link the CBL family to RAS pathways. The recent directed knockout of CBL in mouse indicates its role as a negative regulator of signaling by ZAP70, a tyrosine kinase whose function in T cells includes RAS activation[56]. While neither loss of UNC-101 nor SLI-1 functions results in a Muv phenotype, loss of both together results in RAS pathway activation. These loci did not arise in screens (e.g. Ref. 48) for the A and B class genes discussed above, suggesting that there are multiple sets of partially redundant negative regulators.

Another negative regulator, *sur-5*, was identified as a suppressor of a *let-60 ras(dn)* mutation[57]. The gene

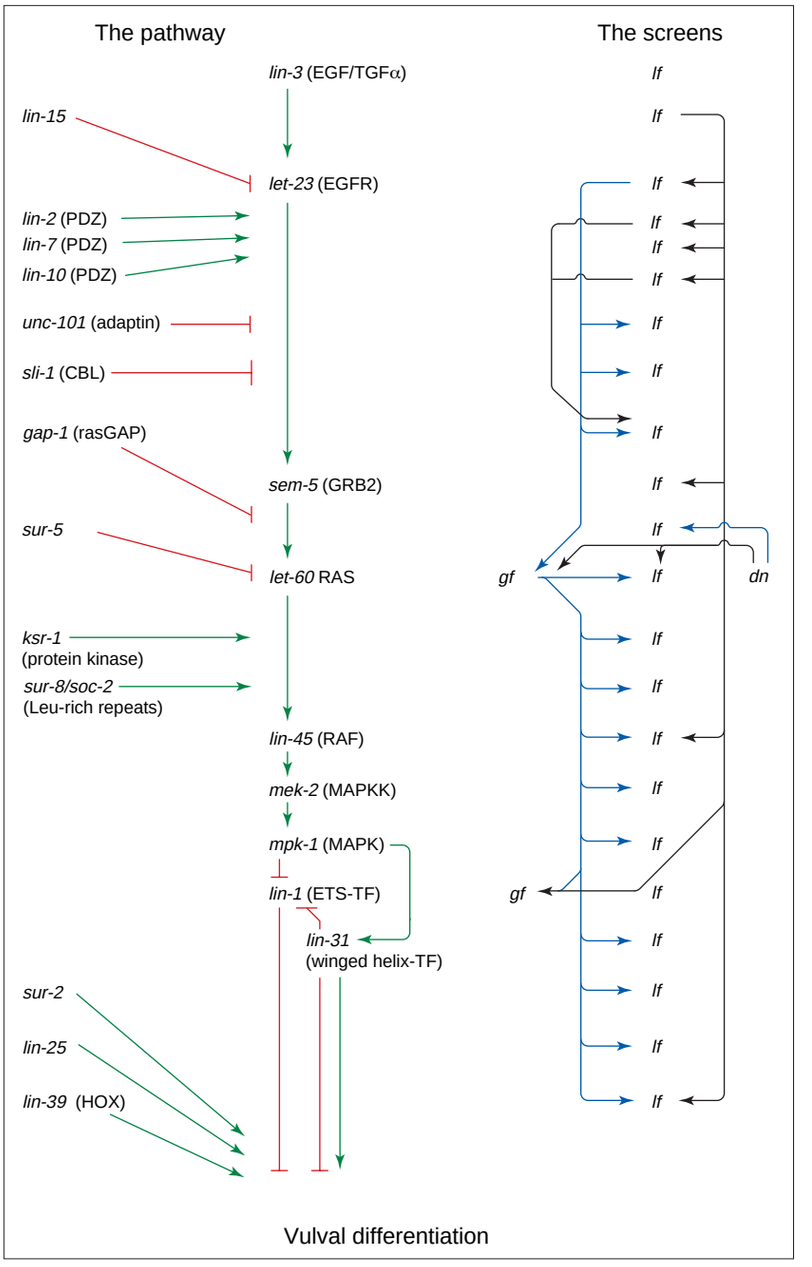

FIGURE 3. Upstream and downstream of LET-60 RAS. The left side depicts the genetic pathway of RAS signaling during vulval induction. Arrows indicate a positive effect. Bars indicate a negative effect. The right side depicts the classes of mutants and many of the suppressor screens that identified those mutations. Abbreviations: *dn*, dominant negative; *gf*, gain-of-function; *lf*, loss-of-function are indicated on the same line as the gene in the pathway diagram. Each curved arrow points from the mutation that was suppressed to the new mutation identified. For example, suppressors of *lin-15(lf)* include *let-23(lf)*, *lin-2*, *lin-7*, etc. The screens are described in the references cited in the text.

sur-5 and its mammalian homolog have been cloned and shown to encode a conserved, novel protein containing potential ATP/GTP- and AMP-binding sites[57]. Consistent with its negative role, overexpressing *sur-5* partially suppresses the *let-60 ras(gf)* Muv phenotype. However, *sur-5* mutations only suppress a subset of *let-60 ras(dn)* alleles, suggesting that these *let-60 ras(dn)* mutations might not exert their dominant inhibitory effects by the same mechanism. For example, different dn proteins might compete with wild-type protein for different activators, a model proposed first from a study of dn RAS mutations in *Saccharomyces pombe* (Ref. 58).

Multiple roles in development

As in mammals and *Drosophila*, RAS as well as many of its upstream regulators and downstream targets have been found to play roles in multiple developmental events in *C. elegans*. Indeed, *let-60* is expressed broadly during *C. elegans* development[59]. As discussed above, the role of *let-60 ras* in development was first described by its mutant defect in vulval development, but as described below, analysis of other functions of *let-60* initiated a molecular understanding of other aspects of development and provided a more general picture of RAS function in development.

Excretory duct cell fate/larval lethality

Null mutations in *let-60* cause early larval lethality[4,6,7]. Besides other cell differentiation events requiring RAS, one cause of such lethality could be failure of many cells to proliferate in the later embryo or in larvae because RAS has been shown to play such a role in mammalian cells. However, mosaic analysis indicates that *let-60* is not essential for general cell proliferation[60]. Such a notion is consistent with a study in *Drosophila* embryos[61]. Assuming that these invertebrate studies can be applied more universally, it is possible that RAS only plays a redundant function in general cell proliferation. Alternatively, while RAS may not play an essential role in normal cell proliferation, activated RAS could play a role in activating cell proliferation in many tissues and cell lines. Mosaic analysis has also revealed the basis of larval lethality in *let-60* mutations[60]. Loss of LET-60 function in a particular cell results in lack of the excretory duct cell, leading to lethality. *C. elegans* has two cells from separated early embryonic lineages that are capable of adopting the excretory duct cell fate, although only one normally does[62]; loss of *ras* activity in both cells eliminates the duct cell fate. Both cells can adopt the duct cell fate in a *let-60 ras(gf)* mutant, suggesting that LET-60 RAS activity specifies the fate of these cells.

Spicule formation

C. elegans males are specialized for mating with hermaphrodites. One major specialization is the generation of the copulatory spicules from a single postembryonic blast cell, B. The spicules are used to locate the vulva, anchor the male cloaca to the vulva during sperm transfer, and to regulate sperm transfer[63]. Mutants defective in the RAS pathway affect male spicule formation[64]. A signal from more anterior cells is required for specification of four of the eight great-great granddaughters of B to generate their correct cell lineages[65]. These four cells generate three different RAS-dependent lineages. As with other RAS effects, decreased activity of the RAS pathway leads to the opposite transformation of cell lineage compared with hyperactivation of the RAS pathway (Refs 64, 66). Consistent with double-mutant analysis, mosaic analysis indicates that *let-60* acts within the B-cell lineage to specify fates[67].

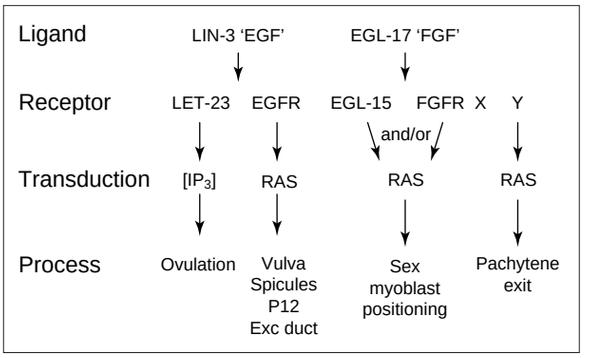

FIGURE 4. Many roles of LET-60 RAS. X and Y are hypothetical receptor tyrosine kinases or other activators of RAS.

Sex-myoblast migration

During the second larval stage, a pair of sex-myoblast cells (SMs) migrate anteriorly from a posterior body region to flank the center of the somatic gonad[68]: this aspect of development has been recently reviewed[69]. A gonad-independent mechanism is sufficient for the initial anterior migration to the mid-body region, and a gonad-dependent mechanism is then required for the precise final positioning of the SMs (Ref. 70). *let-60 ras* appears to play a role in the gonad-dependent process: mutations that reduce RAS function cause defects in the final position of the SMs (Ref. 71) and enhance the SM migration phenotype of mutations in genes acting in the gonad-independent pathway[72]. Mosaic analysis demonstrated that *let-60 ras* is required within the SMs to control the proper positioning, suggesting that a gonadal signal could stimulate RAS activity in SMs (Ref. 71). EGL-17 FGF and EGL-15 FGFR, both of which were shown to play important roles in gonad-dependent SM positioning[3,68,73], might act partly through LET-60 RAS (Fig. 4; Refs 71, 72). By contrast, there is no evidence that either the growth factor LIN-3 nor the EGF-receptor LET-23 functions directly in SM migration (Fig. 4), although they induce expression of *egl-17* in vulval cells[3]. Mutations in *sem-5* have a similar mutant defect as that of *let-60 ras* mutations and, thus, probably acts upstream of RAS for this function[39,72].

Germ-cell development

Many mutations in RAS pathway components also cause a sterile phenotype. The sterile phenotype of *let-60 ras* and its downstream kinases is due to defects in meiotic cell-cycle progression[26]. Specifically, lf mutations in *let-60 ras* as well as in *mek-2* and *mpk-1* cause a failure of meiotic germ cells to exit from pachytene to produce oocytes and sperm. Mosaic analyses of *let-60 ras* and *mpk-1* indicate that these genes exert their functions within the germline[26,67]. Recently, genetic analysis also suggests that *let-60 ras* functions downstream of protein tyrosine phosphatase PTP-2 in oogenesis: an activated *let-60(G13E)* mutation suppresses the oogenesis defect of a *ptp-2* mutation[47]. Such a role for RAS might not be directly observed in *let-60 ras* lf mutations because of earlier

effects (larval lethality and mitotic germ cell defect) of these mutations that will arrest the animal prior to oogenesis.

Mutations of *lin-3 EGF* and *let-23 EGFR* also cause a sterile phenotype, a defect in ovulation of oocytes (Ref. 74; J. McCarter *et al*., pers. commun.). This defect is later in germ line development than the *let-60 ras* sterility, and is independent of the Ras pathway[10,55,75]. For example, *let-60(G13E gf)* suppresses the vulvaless and lethal but not the sterile phenotypes of *let-23* mutations (also see below).

P12 specification

The RAS pathway is also involved in another cell-fate decision, specification of the P12 lineage versus the P11 lineage in the pre-anal ganglion. Both lineages generate three neurons and each generates a specific type of epidermal cell. Activity of RAS is necessary for P12 fate specification, assayed by the fate of its posterior daughter, P12.p. Specification of P12 requires activity of the RAS pathway in response to LIN-3 and LET-23 and is negatively regulated by *lin-15* (Refs 42, 76, 77). The HOX gene *egl-5* is activated in response to RAS signaling and it is necessary and sufficient to specify P12 fate[77,78]. In addition, WNT signaling (via LIN-44 WNT and its putative receptor LIN-17) is necessary for complete P12 specification; it is not clear exactly where the WNT signal is integrated into the RAS pathway[77,79]. A β-catenin homolog, BAR-1, regulates the HOX gene LIN-39 in VPCs (Ref. 80), implicating WNT signaling in HOX regulation in the vulva as well as in P12. Convergence of RAS and WNT signaling pathways on HOX gene regulation might be a general theme in development.

HOX genes and signaling specificity

The roles of three *C. elegans* HOX genes in ventral epidermal cell fate specification have been elucidated. LIN-39 acts in the VPCs P3.p–P8.p; MAB-5 acts in the posterior two VPCs (P7.p and P8.p) to decrease RAS signaling and in the male pre-anal ganglion (PAG) precursors (P9.p–P11.p) to specify their fate. PAG fate specification might involve RAS signaling based on observation that activation of LET-23 by loss of LIN-15 function results in cell fate alterations (P. Sternberg, M. Herman, E. Ferguson and R. Horvitz, unpublished). In the VPCs, LIN-39 is necessary for a response to RAS (Refs 81, 82). Expression of LIN-39 increases in response to RAS activation; both RAS and LIN-39 are necessary for this increase[82]. Strikingly, Maloof and Kenyon[82] demonstrated that substitution of LIN-39 for MAB-5 causes male PAG cells to express hermaphrodite vulval like characteristics, and conversely, MAB-5 misexpression in LIN-39-deficient VPCs results in PAG-like characteristics. These results implicate HOX genes in providing specificity to ventral epidermal cells in response to RAS. Because *egl-5* is transcribed in response to LIN-3 (and presumably RAS) in P11/P12 cells, the specific response to RAS in these cells might also reflect specific HOX gene expression.

Overview

Studies in *C. elegans* of RAS-mediated signal transduction has helped define a signal transduction pathway from transmembrane tyrosine kinases to RAS to nuclear transcription factors, in common with mammals and insects. Although many key proteins, such as the EGF receptor and RAS, have been studied in mammalian cells to elucidate their roles in cell proliferation and differentiation, classical genetic studies of similar genes in *C. elegans* and *Drosophila* have provided valuable information about the functions of these common factors in developmental pattern formation and fate specification. Sensitive genetic screens using specific developmental events, such as vulval development in *C. elegans* or eye development in *Drosophila*, have been used to dissect the signaling pathways and discover genes that have eluded biochemical screens in mammalian cell systems. Specifically, involvement of the following conserved proteins in the RAS pathway were discovered first by genetic screens: SEM-5 (GRB2); SLI-1 (cbl); UNC-101 (adaptin medium chain AP47); LIN-2; LIN-7; KSR1 (mKSR); SUR-5 (hsSUR-5); and SUR-8/SOC-2 (hsSUR-8/SHOC-2). Other genes, such as SUR-2, LIN-25, LIN-15A and LIN-15B, are not yet represented in mammals. If they are, then the analysis of their function in *C. elegans* will probably have predictive power.

Transcription factors have emerged as key mediators of RAS signaling specificity. As discussed above, the HOX genes contribute to the different effects RAS has in ventral epidermal precursors, which differ in their response to RAS along the body axis. Also, the winged helix protein LIN-31 contributes specificity to its ETS domain partner, LIN-1. Defining the cellular role of these factors will require identification of some of their target genes.

Many of the RAS functions described are coupled to LET-23 functions (Fig. 4). However, LET-23 also regulates inositol polyphosphate signaling independently of RAS (Ref. 75). Distinct tyrosine residues, defining potential SH2 binding sites in the cytoplasmic domain of LET-23, mediate specific activation of either the inositol phosphate or the RAS pathway[83]. Thus, one way in which one receptor tyrosine kinase has tissue-specific effects is by activating distinct signaling pathways. On the other hand, RAS does more than respond to LET-23 (Fig. 4). RAS might respond to the receptor EGL-15 in sex myoblast positioning, and possibly a second receptor, 'X'. Also, during oogenesis, RAS probably responds to yet another receptor tyrosine kinase, labelled 'Y' in Fig. 4. The studies of PTP-2 suggest an additional function for RAS, possibly in this oogenesis pathway. A theme that has clearly emerged from these studies in *C. elegans* is that RAS plays a major role in specifying cell fate during development, rather than controlling cell proliferation. Now that we have a reasonably detailed understanding of the RAS pathway, we can also look forward to addressing whether and how other cell regulatory pathways interact with RAS to regulate development.

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